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SciVerse ScienceDirect
Journal of Sport and Health Science 2 (2013) 94–100

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Review

Exercise and antioxidant supplements in the elderly

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Received 28 November 2012; revised 4 February 2013; accepted 4 March 2013

Abstract

Both exercise and aging increase reactive oxygen species (ROS), which can result in damage to cells. Aging is the result of damage caused by ROS to the mitochondrial genome in post mitotic cells and numerous studies have demonstrated an increase in ROS or their byproducts with exercise. ROS can cause oxidative stress as they overwhelm the antioxidant cellular defenses. Therefore interventions aimed at limiting or inhibiting ROS production, such as supplementation with antioxidant vitamins, should be able to reduce fatigue during muscle contraction and the rate of formation of aging changes with a consequent reduction of the aging rate and disease pathogenesis. However, it has been shown that ROS are essential signaling molecules which are required to promote the health benefits of exercise and longevity. In young individuals, ROS are required for normal force production in skeletal muscle, for the development of training-induced adaptations in endurance performance, as well as for the induction of the endogenous defense systems. Thus, taking antioxidants during training, in young athletes, seems to be detrimental. However, antioxidant supplementation may be expected to be beneficial and is receiving growing attention in the active old population. In this manuscript we review the literature associated with the main areas of interest in this topic.

Keywords: Adaptations; Aging; Antioxidant enzymes; NF-κB; Oxidative stress; PGC-1α; Skeletal muscle

1. Free radicals and exercise

Skeletal muscle generates reactive oxygen species (ROS) during contractile activity. Research in this area started in 1954 when the emerging technology of electron spin resonance (ESR) spectroscopy was used to generate the first data showing that free radicals are present in muscle.[1] However, first suggestion that exercise was associated with an increase in lipid peroxidation byproducts did not appear until the late 1970s.[2] The biological importance of this finding was unclear at the time. It was not until the early 1980s that researchers identified the first link between muscle function and free radical biology. ESR was again used to show that free radical content is elevated in isolated frog limb muscles stimulated to contract repetitively.[3] Shortly afterward, a ground-breaking report was published showing a 2- to 3-fold increase in free radical content of skeletal muscle from rats run to exhaustion.[4] These findings were associated with three aspects of damages that are now well-recognized: increased lipid peroxidation, decreased control of mitochondrial respiration, and decreased integrity of the sarcoplasmic reticulum. The same study showed that vitamin E deficiency inflated these three changes, indicating exercise-induced changes were sensitive to both free radical production and antioxidant buffering.[4] Since then, research in the area has grown rapidly. It is now clear that

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Peer review under responsibility of Shanghai University of Sport

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jshs.2013.03.007

intense muscular contractile activity can result in oxidative stress not only in animals but also in humans. For instance, during the Tour de France, cyclists shown significant increases in plasma malondialdehyde (MDA) levels,[5] whereas similar results have been found in athletes after a marathon running.[6]

There are several potential tissue sources from which ROS may be produced during exercise: heart, lungs, white blood cells and skeletal muscle have been most studied.[7,8] At the subcellular level, several sources of free radicals have been studied in skeletal muscle during exercise.[9] It has generally been assumed that an increase in oxygen consumption by mitochondria would lead to an increase in $O_2^{\bullet -}$ formation from complexes I and III. However, recent research suggests that mitochondria may not be the dominant source of ROS during exercise.[9,10] Rigorous exercise, especially eccentric contractions, may generate ROS via nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase from neutrophils and sarcolemma and secondarily, via myeloperoxidase. Interestingly, superoxide anions generated by these enzymes have been shown to regulate contractile function via calcium release in the cardiac muscle.[11] Phospholipase A2, an important enzyme involved in the metabolism of membrane polyunsaturated fatty acid during inflammation, has been identified as a modulator of cytosolic oxidant production in skeletal muscle.[12] Nethery et al.[13] showed that phospholipase A2 function is essential for the rise in intracellular ROS that occurs during repetitive, fatiguing contractions. Cyclooxygenase and lipoxygenase are involved in ROS production with phospholipase A2. The role of xanthine oxidase (XO) in oxidant generation during high-intensity intermittent exercise has long been recognized.[6,14,15] Depletion of ATP during demanding muscle contraction results in an accumulation of hypoxanthine and xanthine and conversion of xanthine dehydrogenase to XO. These conditions set the stage for generating $O_2^{\bullet -}$ when oxygen is replenished to relatively hypoxic muscle.[16] Administration of allopurinol or oxypurinol, a drug widely used in the clinical practice to treat gout due to its inhibitory effect on XO, has been shown to decrease muscle oxidative stress after exhaustive exercise both in humans and in rats.[5,17,18] Finally, nitric oxide (NO) is generated continuously within skeletal muscle by NO synthase (NOS) with an important function to regulate vascular smooth muscle tone.[19] Heavy muscle contraction can increase NO production via activation of eNOS or iNOS which may have some detrimental effect due to the danger of forming highly reactive peroxynitrite.[20] A schematic diagram of the sources of free radicals in skeletal muscle is in Fig. 1.

2. The role of antioxidants in the modulation of skeletal muscle adaptations to exercise

As mentioned in the previous section free radical production during muscle contraction has been related to several aspects of damage. Thus, the idea of the deleterious effects of free radicals has been firmly entrenched in the minds of scientists during last 30 years.[10] It has been generally accepted that increasing the intracellular levels of antioxidants within a muscle cell should provide protection against these oxidizing agents and reduce fatigue.[21–23] During the early 1980s, several research groups investigated the role of antioxidant nutrients in the protection of cells and organelles from radical-mediated oxidative damage.[24] In 1983 Jackson et al.[25] examined the role of ROS as damaging agents to muscle and the possible beneficial effects of vitamin E in reducing exercise-induced damage. These studies stimulated the interest of many laboratories to investigate whether antioxidant nutrients could retard both tissue damage and muscle contractile dysfunction that occurred during some forms of muscular exercise. There is no doubt that antioxidant supplementation decreases the markers of oxidation in tissues.[26–28] Based on these data many athletes consume quantities of vitamins E and C well above the recommended dietary allowances.[29] Vitamin C is one of the biggest-selling nutrients in the U.S. vitamin and mineral market, with predominantly healthy people (including athletes) topping the buyers' list.[30] However, the positive effects of dietary antioxidants against contraction-induced muscle damage and muscular fatigue are not commonly observed.[7] Although the generation of ROS is an inevitable event associated with muscle contraction during physical exercise, we now know that its production is determined by the intensity, frequency, and duration of the exercise protocols. It has been shown that exercise training reduces the oxidative stress of

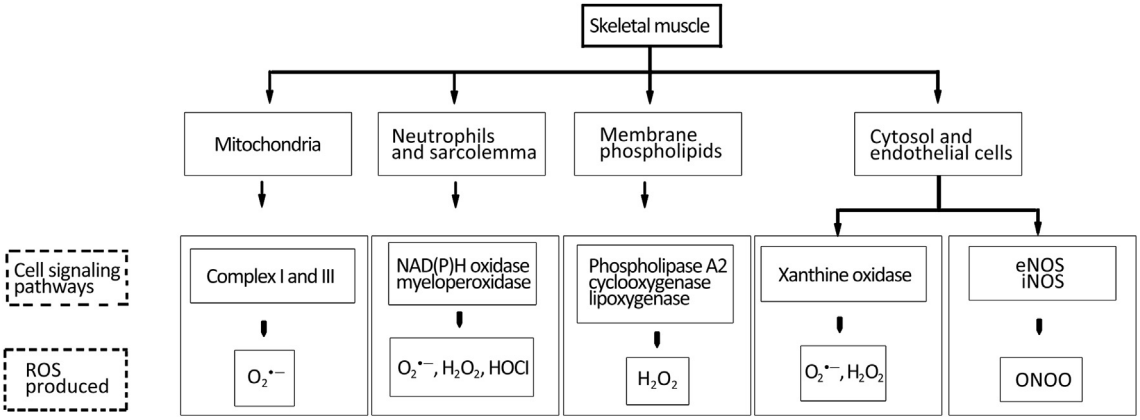

Fig. 1. Potential sites for the production of free radicals in skeletal muscle. There are several sources of free radicals in skeletal muscle. They are located in mitochondria, cytosol, sarcolemma, and endothelial cells. Abbreviations: ROS = reactive oxygen species; NOS = nitric oxide synthase; NAD(P)H = nicotinamide adenine dinucleotide phosphate; ONOO = peroxynitrate.

exercise, trained athletes show less evidence of lipid peroxidation for a given bout of exercise and an enhanced defense system in relation to untrained subjects.[10] Thus exercise training can be considered as an antioxidant.[10] The dramatic ability of the body to increase antioxidant capacity with acute and chronic exercise has been described in several tissues.[31–33] There is now an appreciation that the ROS generated during muscle contraction have a physiological role in the adaptations to exercise. In response to the free radical assault, the cell has developed a number of antioxidant defense systems. There is growing evidence that the continued presence of a small stimulus such as low concentrations of ROS is in fact able to induce the expression of antioxidant enzymes and other defense mechanisms. The basis for this phenomenon may be encompassed by the concept of hormesis,[34] which can be characterized as a particular dose–response relationship in which a low dose of a substance is stimulatory and a high dose is inhibitory. In this context radicals may be seen as beneficial as they act as signals to enhance defenses rather than deleterious as they are when cells are exposed to high levels of these radicals. Recently the hormesis theory has been extended to the ROS generating effects of exercise.[35,36] In skeletal muscle hydrogen peroxide at a low concentration increases calcium release from the sarcoplasmic reticulum and force production, whereas a massive increase in hydrogen peroxide concentration results in a sharp decrease in force output.[37] Animals frequently exposed to exercise (chronic training) have shown less oxidative damage after exhaustive exercise than untrained ones. This is largely due to the up-regulation of endogenous antioxidant enzymes such as mitochondrial superoxide dismutase, glutathione peroxidase, and γ-glutamylcysteine synthetase.[38] We have shown that this up-regulation is mediated by redox sensitive transcription factors such as nuclear factor κB (NF-κB).[6,17,39] Thus, the convenience of supplementing antioxidant vitamins in the sport population is nowadays an object of debate. In fact training studies conducted to determine whether antioxidant vitamins improve exercise performance have generally shown that supplementation is useless[40–44] or even negative.[45] Several studies suggest that antioxidants may have detrimental effects on performance.[46–49] We have found that vitamin C supplementation decreases training efficiency because it prevents exercise-induced mitochondrial biogenesis.[50] These results have been confirmed by other research groups.[51,52] A large proportion of athletes, including elite athletes, take vitamin supplements, often large doses, seeking their beneficial effects on performance.[53] The complete lack of any positive effect of antioxidant supplementation on physiologic and biochemical outcomes consistently found in human and animal studies raises questions about the validity of using oral antioxidant supplementation in the sport population.[45]

3. Free radicals and exercise at old age

There are many theories of aging.[54] One of the most prominent theories to explain aging is the free radical theory of aging which was initially proposed by Harman[55] in the 1950s. It proposes that free radicals derived from oxygen are responsible for damage associated with aging. The antioxidant systems are unable to counterbalance all the free radicals continuously generated during the life of the cell. This results in oxidative damage in the cell and thus in tissues. There is a great deal of experimental proof in support of this theory. The findings in the laboratory of Britton Chance that ∼2% of oxygen consumed by mitochondria in state 4 is converted to hydrogen peroxide underlined the role of mitochondria in ROS production.[56] These experiments led to Jaime Miquel to refine the free radical theory of aging and in the 1970s he formulated the mitochondrial free radical theory of aging. The main contributions of Miquel were: emphasized the importance of mitochondrial DNA as a target of oxidants produced during aging, and pointing out that mitochondrial biogenesis might be impaired in aging.[57]

The mitochondrial theory of aging, although recently questioned,[58] has been tested in various laboratories and there are many published papers in support of this theory.[59,60] The continuous free radical generation by mitochondria during the whole lifespan, causes a chronic oxidative stress that plays a critical role in aging. Thus, aging is associated with free radical generation in several tissues including skeletal muscle.[55,61] Senile sarcopenia is defined as the loss of muscle mass and force associated to aging.[62] It has been estimated that muscle fibre loss occurs as early as at age 25 and that at age 80 total muscle fibre number shows a decrease of almost 40%.[63] ROS have been proposed to be involved in the underlying mechanism of age-induced sarcopenia. As a response to this oxidative stress skeletal muscle antioxidant enzyme activities are increased with old age.[64] However, protein and mRNA levels of these enzymes are found to be either decreased or unaltered in the aged muscle.[65] Alterations in the NF-κB cell signaling pathway seem to be responsible for this impairment. NF-κB is believed to be constitutively activated in skeletal muscle at old age. This increased transcription seems to be part of a general cellular adaptive response aimed at providing protection against subsequent, damaging insults.[66] However, chronic activation of NF-κB leads to the higher basal expression of pro-inflammatory cytokines, chemokines, and adhesion molecules. In fact, it has been identified as a main etiological reason for aged-related muscle wasting and sarcopenia.[67] However, there is a failure to fully activate NF-κB in the skeletal muscle of old animals following contractile activity[66] (Fig. 2). The mechanisms responsible for this fall are unclear. Thus, during aging there is an impairment in the signal transduction of antioxidant gene expression in response to oxidative stress.[68]

Other relevant co-activator affected by aging is peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). It acts as a master regulator of energy metabolism and mitochondrial biogenesis by coordinating the activity of multiple transcription factors.[69] Aging has been associated, in skeletal muscle, with reductions in mitochondrial oxidative phosphorylation activity, mitochondrial DNA mutations, reductions in mitochondrial DNA content, decreased activities of the mitochondrial electron transport chain, and altered apoptotic

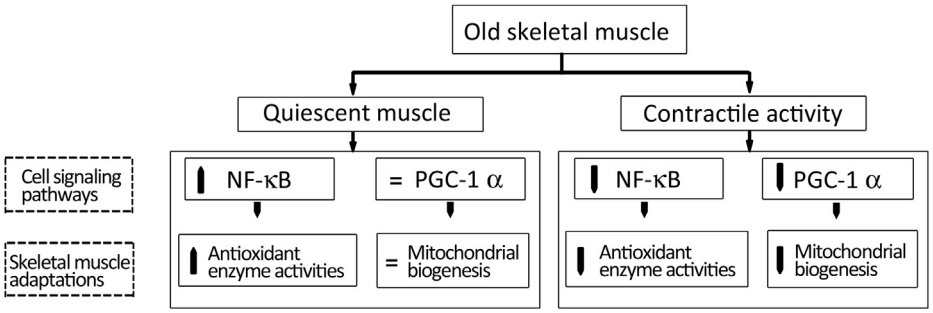

Fig. 2. Redox sensitive cell signaling pathways altered in skeletal muscle at old age. Nuclear factor κB (NF-κB) is constitutively activated in skeletal muscle at old age which leads to an impairment in the oxidative stress response. However, there is a failure to fully activate NF-κB in the skeletal muscle of old animals following contractile activity. The attenuated mitochondrial biogenesis reported in both the quiescent and stimulated skeletal muscles at old age compared to young is at least partially due to an attenuation of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) signaling ability.

signaling.[70] Thus, the promotion of mitochondriogenesis is critical to prevent aging in skeletal muscle. We have recently shown that muscle from old rats present a marked loss in mitochondriogenesis and that this may be due to a lack of induction of PGC-1α.[71] We found a striking similarity between the response to exercise training in *PGC-1α* knockout mice and in old rats. In young rats, PGC-1α was activated in skeletal muscle not only by training but also by cold exposure or triiodothyronine. However, in the old animals we found an age-associated lack of induction of PGC-1α in response to exercise training or to any of the other stimuli tested in rat skeletal muscle. Our study highlighted the importance of maintaining a normal PGC-1α responsiveness to maintain normal muscle function (Fig. 2).

4. The role of antioxidants in the modulation of the aging process

An important characteristic of the free radical theory of aging is that, it opens up room for intervention, because if radicals are causing oxidative damage to cells and this is associated with age-associated damage, then administration of antioxidants could delay aging and perhaps even prevent age-associated diseases. Cutler[72] observed that several antioxidants such as vitamin E, uric acid, cellular plasmin, or superoxide dismutase in several organisms show an inverse relationship with the basal metabolic rate and with the maximal longevity of the species. Thus, he proposed that the maximal lifespan should be correlated with the antioxidant capacity of cells. In keeping with this line of thought Orr and Sohal[73] observed that double transgenic *Drosophila* over-expressing Cu/Zn-superoxide dismutase and catalase show less oxidative stress and longer lifespan, both mean and maximal. Moreover, they found that the process of aging was slowed. Indeed, the transgenic *Drosophila* showed a lower loss in physical activity and less markers of damage in proteins. However, the assumption that antioxidant supplements are in general good for one's health has been proof to be wrong. A critically important point is the relationship between the various antioxidants in cells. Persons with defects in absorption of vitamin E or with low glutathione levels show different conditions but not an accelerated aging. In fact, using high doses of vitamin E in age-related diseases such as Alzheimer's, has been questioned after the publication of some studies which show that its administration is detrimental for the patients.[74] The evidence on the detrimental effects of antioxidant supplementation when given to patients and healthy people is robust. In 2007, Bjelakovic et al.[75] looked at data from 67 studies on antioxidant supplements and they concluded that beta carotene, vitamin A, and vitamin E supplementation seemed to increase the risk of death. This data confirmed previous reports showing that long-term vitamin E supplementation may increase the risk for heart failure in patients with vascular disease or diabetes mellitus.[76]

5. Exercise and antioxidant supplementation at old age

The beneficial effect of physical activity for the promotion of health and curing of diseases among individuals of all ages is beyond all doubt. Strong scientific evidences link physical activity to several benefits, including the promotion of health span and not only of lifespan. Although physical activity has many well-established health benefits,[77] aging and strenuous exercise are associated with increased free radical generation in the skeletal muscle.[78] Thus, whether exercise would worsen the skeletal muscle oxidative stress in aged population has been an object of debate. Research evidence indicates that senescent organisms are more susceptible to oxidative stress during exercise because of the age-related ultrastructural and biochemical changes that facilitate ROS generation.[78] Aging also increases the incidence of muscle injury, and the inflammatory response can subject senescent muscle to further oxidative stress. Furthermore, muscle repair and regeneration capacity is reduced at old age that could potentially enhance the cellular oxidative damage.[78] Thus, several researchers consider that dietary antioxidant supplementation should be beneficial in the old physically active population.[79] Recent studies suggested a beneficial relationship between antioxidant vitamin (e.g., vitamin C) intake and physical performance in elderly people.[80] It has been shown that intake of resveratrol, together with habitual exercise, is beneficial for suppressing the aging-related decline in physical performance.[81] Moreover

it has been shown that antioxidant supplementation improves indices of oxidative stress associated with repetitive loading exercise and aging and improves the positive work output of muscles in aged rodents.[82] Bobeuf et al.[83] found that 6 months of resistance training combined with antioxidant supplementation significantly increased fat-free mass in older adults. However, these results have not been confirmed by other studies. Nalbant et al.[84] found that 6 months of vitamin E supplementation had no additive effect beyond that of aerobic training on indices of physical performance and body composition in older sedentary adults. Regarding bone density it has been shown that combination of resistance training with antioxidant vitamins supplementation does not seem to produce synergistic effects on the prevention of osteoporosis.[85] The convenience of supplementing with antioxidant vitamins in the old sport population is nowadays, as in the young population, an object of debate. Richardson's research group identified a clinically significant paradoxical cardiovascular response to exercise training and antioxidant supplementation in the elderly.[86] Antioxidant administration, after exercise training, blunted training-induced reduction in blood pressure as well as the exercise-induced improvements in flow-mediated vasodilation. The paradoxical effects of these interventions suggest a need for caution when exercise and acute antioxidant supplementation are combined in elderly mildly hypertensive individuals. Thus, the paradoxical effects of antioxidant supplementation, when combined with exercise training, reveal an intriguing, but complex, relationship between aging, exercise, and oxidative stress. More research for a better clarification of the field is required.

Acknowledgments

This work was supported by grants SAF2010-19498 from the Spanish Ministry of Education and Science (MEC (gs1)); ISCIII2006-RED13-027 from the Red Temática de investigación cooperativa en envejecimiento y fragilidad (RETICEF (gs2)); P2011/02 RM from Cátedra Real Madrid-UEM (gs3); PROMETEO2010/074 from Conselleria de Sanitat de la Generalitat Valenciana (gs4); 35NEURO GentxGent from Fundació Gent Per Gent de la Comunitat Valenciana (gs5) and EU (gs6) Funded COSTB35 and CM1001. This study has been co-financed by funds from the European Union.

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